

REMARKS

Reconsideration of the rejections set forth in the Office action mailed October 25, 2006 is respectfully requested, for the reasons discussed below. Claims 1-3, 5-11, and 19-27 are currently pending.

I. Allowable Subject Matter

Claims 7-9 and 11 were subject to no rejections, but were objected to as being dependent on a rejected base claim.

II. Rejections under 35 U.S.C. §102(b)

Claims 1-3, 5-6, 10, 19-20, 23-24 and 27-27 were rejected under 35 U.S.C. §102(b) as being anticipated by Bruice *et al.*, U.S. Patent No. 5,686,242. This rejection is respectfully traversed for the following reasons.

A. The Claims

Independent claim 1, as presented above, is directed to a method of separating a population of duplexes, each comprising one of a population of different, substantially uncharged oligomeric analyte molecules and a specific probe molecule,

wherein the substantially uncharged analyte molecules are oligonucleotide analogs composed of linked subunits of which at least 90% are uncharged, and the specific probe molecule is a fully charged nucleic acid or fully charged nucleic acid analog,

the method comprising:

(a) applying to an ion exchange medium a mixture of (i) the different substantially uncharged analyte molecules and (ii) the specific probe molecule, under conditions such that the probe molecule forms stable duplexes with a plurality of or all of the different substantially uncharged analyte molecules,

thereby forming a plurality of different probe-analyte duplexes, which differ from each other with respect to the presence, length or position of an unhybridized portion of the probe molecule, and

(b) separating the different probe-analyte duplexes from each other and from single stranded analyte or probe molecules within the medium.

B. The Cited Art

Bruice *et al.* describe a method of assaying pools of oligonucleotides, of which at least a portion of the sequences are selected randomly, for binding to a target. A preferred methodology employs “solid support based affinity chromatography wherein the target molecule is stably attached to a solid support and the ligands [e.g., oligonucleotides] are in the flowing mobile phase”; alternatively, “both the target molecule and the ligand [oligonucleotide] pool are in the mobile phase” (column 12, lines 48-55). The reference emphasizes the separation of active (that is, binding) oligonucleotides from inactive oligonucleotides. See, for example, column 3, lines 36-37, column 4, lines 6-7, column 10, lines 58-59, column 11, lines 25-26, column 12, line 23, column 13, line 7, etc.

The Examiner points to the disclosure at column 22 (Example 12) as allegedly anticipating the applicants’ broad claim. However, this disclosure fails to meet several limitations of the broad claim, as follows.

(1) The molecules making up the alleged group of “different, substantially uncharged oligomeric analyte molecules” are not “substantially uncharged”, as claimed.

The Examiner alleges (page 3 of Office Action) that the “2’-O-methyl-oligonucleotides” disclosed at column 22, lines 26-29 of the reference are “substantially uncharged oligomeric analyte molecules”.

In accordance with standard nomenclature, a “2’-O-methyl” oligonucleotide has a methyl substituent on the sugar ring. There is no indication of any other structural modification to these molecules in the reference. The reference defines an “oligonucleotide” as “a polynucleotide formed from naturally occurring bases and furanosyl groups joined by native phosphodiester bonds” (column 5, lines 40-43). Therefore, the “2’-O-methyl-oligonucleotides” must be assumed to have “native phosphodiester” linkages and therefore are fully charged oligonucleotides.

(2) The molecules are not applied “to an ion exchange medium”, as claimed.

The column to which the molecules are applied in the reference Example is a “Superous [sic - Superose®] 12 HR 10/30” fast protein liquid chromatography column (column 22, lines

18-20). As shown in the enclosed product information sheet (see table on second page), this column is comprised of cross-linked agarose (a polysaccharide) and is used for gel filtration chromatography, which is based on molecular size. (See the definition below.) Also note that ion exchange columns are listed in a separate category from Superose[®] gel filtration columns on the last page of the enclosed document.

Therefore, the molecules are not applied “to an ion exchange medium”, as claimed.

gel filtration <molecular biology> An important method for separating molecules according to molecular size by percolating the solution through beads of solvent permeated polymer that has pores of similar size to the solvent molecules. ... Typical gels for protein separation are made from polyacrylamide or from flexible (Sephadex) or rigid (agarose, Sepharose) sugar polymers. The size separation range is determined by the degree of cross linking of the gel.

On-Line Medical Dictionary

Published at the Centre for Cancer Education, University of Newcastle upon Tyne

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In view of items (1) and/or (2) above, the reference clearly does not anticipate the claims.

Applicants further observe that:

(3) There is no indication that duplexes of the different oligonucleotides with the target RNA (ras 47-mer stem/loop sequence) were separated from each other on the column. The reference indicates that the FPLC column was “calibrated to separate *bound from unbound oligonucleotides*” (column 22, lines 19-20; emphasis added in all citations). The mixture of the oligonucleotides and RNA is run to determine “retention times of *bound* and differential rate *dissociated* species” (column 22, lines 44-45). The mixture is “loaded on the column and run to provide an elution profile” (column 22, lines 45-46). However, the elution profile is not shown or described. There is no evidence that separation of different duplexes on the column occurred.

It does not appear, in fact, that the authors sought to separate different duplexes on the column. In discussing techniques for separation, the reference states that “Forces effecting differential transport of *bound target and free ligands* is all that is required” (column 12, lines 55-57).

Furthermore, the subsequent working example (Example 13) describes the use of the

FPLC column calibrated in Example 12 to assay a “random” group of oligonucleotides for binding to the ras RNA. In that process, the “RNA-oligonucleotide(s) bound complex(es) were recovered using biotin-streptavidin capture of the biotinylated ras RNA”; then the “bound oligonucleotide(s)/ras RNA complex(es) are dissociated...and the selected oligonucleotide(s) recovered”. Thus, the RNA-oligonucleotide “complex(es)” are recovered as a group, not separately, even when “random” oligonucleotides are used. If this is the case, it is unlikely that “complex(es)” based on highly similar oligonucleotides (i.e., the “calibrating” oligonucleotides used in Example 12) would be separated from each other.

Since the reference does not disclose all of the elements set out above in claim 1 and its dependent claims, the claims cannot be anticipated by this reference under 35 U.S.C. §102(b). In view of this, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §102(b).

III. Rejections under 35 U.S.C. §103(a)

Claims 21-22 and 24 were rejected under 35 U.S.C. §103(a) as being unpatentable over Bruce *et al.*, cited above, in view of Ecker *et al.*, U.S. Patent No. 5,986,053, cited in a previous office action. This rejection is respectfully traversed for the following reasons.

A. The Claims

Dependent claims 21-22 recite that the analyte molecules of claim 1 are morpholino oligomers. Dependent claim 24 recites that the probe molecule of claim 1 is DNA.

These claims otherwise include all the limitations of parent claim 1, discussed above.

B. The Cited Art

Ecker *et al.*, “Peptide Nucleic Acids Complexes of Two Peptide Nucleic Acid Strands and One Nucleic Acid Strand”, describes gel motility shift assays in which a PNA is hybridized with complementary DNA, both in single stranded and duplex form, where the DNA target may be labeled.

The Examiner states that it would have been obvious to substitute labeled DNA for RNA in the method of Bruce *et al.*, or to substitute phosphoramidate-linked morpholino oligomers (which are not discussed in Ecker *et al.*) for the 2'-O-methyl oligonucleotides in the method of Bruce *et al.*

However, even if these substitutions were made, the reference disclosures would not suggest that duplexes of different oligomeric molecules with a particular RNA or DNA could be separated from each other on, for example, an ion exchange column.

As outlined above, the primary reference does not indicate that the authors achieved, or even sought, such separation. In discussing techniques for separation, Bruice *et al.* states that "Forces effecting differential transport of *bound target and free ligands* is all that is required" (column 12, lines 55-57).

As discussed above, Example 13 states that "RNA-oligonucleotide(s) bound complex(es)", derived from random oligonucleotides, were recovered as a group, via streptavidin capture, and dissociated.

Similarly, two Examples employing separation by electrophoresis (Examples 9 and 10) describe "separation of bound vs. unbound material" (column 19, line 39; column 21, line 32), whereupon the "bound complex is excised from the gel" (column 19, line 47; column 21, lines 40-41). In these examples as well, there is no suggestion whatsoever that different complexes were separated from each other on the support medium.

Accordingly, the applicants request that the rejection of these claims under 35 U.S.C. §103(a) be withdrawn.

IV. Conclusion

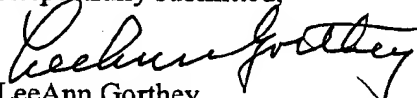
In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the view of the Examiner a telephone consultation would expedite allowance of the pending claims, the Examiner is encouraged to call the undersigned at the number provided.

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Respectfully submitted,


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Superose® 6 HR 10/30 Superose® 12 HR 10/30

INSTRUCTIONS

Columns prepacked with Superose® are designed for high performance gel filtration of proteins, peptides, polynucleotides and other biomolecules.

Introduction

Gel filtration columns of Superose have been designed for operation with FPLC® System. These instructions will help you obtain the best results from your column.

Unpacking

Please check the delivery against this list:

Designation	Code No.	No. supplied
Superose 6 HR 10/30 or	17-0537-01	1
Superose 12 HR 10/30	17-0538-01	1
Wrench	19-7481-01	1
Filter kit HR 10	18-3575-01	1
Filter tool	18-3590-01	1
Instructions		

Quality control tests

To guarantee the quality of Superose 6 HR 10/30 and Superose 12 HR 10/30, each column is tested for its efficiency. Each media batch undergoes a function test to ensure reproducible results.

Connecting the column to FPLC System

1. The column is supplied with rubber tubing connecting the outlet to the inlet of the column. Remove this tubing and connectors.
2. Connect the shorter preflanged tubing (the outlet) to the detector.
3. Connect the longer preflanged tubing (the inlet) to a valve which is used for sample injection and elution e.g. Amersham Biosciences Valve V-7 or Motor Valve MV-7. When using the Valve PV-7 or Motor Valve PMV-7, connect the Union, M6 female/1/16" male (Code No. 18-3858-01) between the flanged tubing and the valve.

Connecting the column to HPLC Systems

Columns prepacked with Superose can be used with any HPLC system if the pump can provide precise and accurate flow at relatively low back-pressures. The column should be connected as described for FPLC System via two unions which adapt the M6 connector to 1/16" tubing (see "Spare parts and accessories").

Important before use

The prepacked columns are stable up to a pressure-drop over the gel bed of 1.5 MPa (15 bar, 215 psi) for Superose 6 and 3 MPa (30 bar, 430 psi) for Superose 12. Operation bed pressure should not exceed 1.2 MPa for Superose 6 and 2.4 MPa for Superose 12.

The gel is delivered in 20% ethanol. This solvent produces high back-pressures and we recommend the following equilibration guidelines to carefully wash out the ethanol.

Superose 6:

Superose 6 swells slightly when transformed from ethanol to water. To avoid local high back-pressures, program the following steps for initial equilibration:

Step	Flow rate	Time
1	0.2 ml/min	60 min
2	0.5 ml/min	120 min

After equilibrium, we recommend a flow rate of 0.1–0.5 ml/min. However, if buffers and samples are rather viscous, you may need to choose a lower flow rate to keep the pressure below 1.2 MPa.

52-1768-00

Edition AK

Superose 12:

Start to equilibrate at a low flow rate (0.2-0.5 ml/min) and keep the pressure below 2 MPa (20 bar, 300 psi) until two column volumes (50 ml) have passed. After equilibration, we recommend a flow rate of 0.5-1 ml/min. However, if buffers and samples are rather viscous, you may need to choose a lower flow rate to keep the pressure below 2.4 MPa.

Note: A precipitation of the sample may block the filter and cause it to compress the gel. To avoid this, we advise you never to set the pressure limit control at more than 0.2 MPa above your actual operating pressure.

Gel properties

Superose is a cross-linked, agarose-based medium optimised for high performance gel filtration of biomolecules. The narrow particle size distribution of Superose enables high flow rates at low back-pressures.

Property	Superose 6	Superose 12
Matrix		
Exclusion limit (globular proteins)	~4x10 ⁷ M _r (approx.)	~2x10 ⁸ M _r (approx.)
Optimal separation range (globular proteins)	5 000–5x10 ⁶ M _r	1 000–3x10 ⁶ M _r
Matrix composition	Composite of cross-linked agarose	
Bead diameter (µm)	11–15	8–12
Prepacked column		
Bed dimensions (mm)	10 x 300–310	10 x 300–310
Bed volume (ml)	24 (approx.)	24 (approx.)
Max bed pressure	1.5 MPa (15 bar, 215 psi)	3 MPa (30 bar, 430 psi)
Operation bed pressure#	1.2 MPa (12 bar, 175 psi)	2.4 MPa (24 bar, 350 psi)
Max flow rate (ml/min)#	1.0	1.5
Recommended flow rate (ml/min)#	0.3–0.5	0.5–1.0
Column efficiency (H ⁻¹)	> 30 000	> 40 000
pH stability		
long term	3–12	3–12
short term	1–14	1–14
Temperatur	4–40 °C	4–40 °C

At room temperature in aqueous buffer

Hydrophobic interactions: Some hydrophobic interactions have been recognized, i.e. some compounds may be eluted somewhat later than predicted. These interactions can be of considerable value to the resolution.

Chemical and physical stability

The columns with Superose are resistant to all solutions commonly used in gel filtration, including 8 M urea, 6 M guanidine HCl and 30% acetonitrile in the temperature range 4–40 °C. With aqueous salt solutions, Superose is stable in the pH range 3–12 during operation and pH range 1–14 for cleaning procedure. All detergents, nonionic or ionic, such as SDS, may be used. Limited degradation of the polysaccharide chains may occur under oxidizing conditions. If the column is used for long periods at pH 1, the gel matrix may be subjected to limited hydrolysis.

The back-pressure should not exceed 1.2 MPa for Superose 6 or 2.4 MPa for Superose 12. The column materials are glass and plastics which are all biocompatible. This means that there is no risk for sample contamination by metal ions.

Eluent and sample preparation

Degas and filter all solutions through a 0.22 µm sterile filter. Water should be of Milli-Q* or corresponding quality. Use HPLC grade solvents, salts, and buffers. Centrifuge (10 000xg for 10 min) or filter samples through a 0.22 µm filter. Be sure to select a **solvent resistant filter** if samples are dissolved in organic solvents. If your sample is of high viscosity, dilute it with the eluent.

Column equilibration

Before applying the sample, equilibrate the column with two column volumes (50 ml) of eluent buffer. Equilibration is not necessary between runs with the same eluent.

Sample application and elution

Make sure the sample is recently filtered or centrifuged before applying it to the column. A prefilter between injector and column is not recommended unless automated injections are performed. A prefilter reduces the resolution by approximately 10%. Flow rates above 0.5 ml/min are not recommended for best resolution.

* Milli-Q is a registered trademark of Millipore Corp.

Some useful eluent compositions

pH	Buffer/Eluent	Properties/applications example
1	70% Formic acid	High solubility for peptides and protein fragments. Low UV-transparency at 214 nm. Not recommended for Superose 6. <i>After having used 70% formic acid wash according to the cleaning steps 6 and 7 below before changing to another eluent</i>
1.4	0.05 M HCl	Good solubility for peptides.
5.0	0.1 M Ammonium-acetate	Good solubility for some enzymes e.g. cellulases. Volatile.
<7	up to 8 M Urea	Good solubility for many components. Biological activity can be maintained at lower urea contents. Certain risk for carbamylation of proteins.
7.2	0.05 M Phosphate 0.15 M NaCl	Physiological conditions
7.8	0.15 M Ammonium-hydrogencarbonate	Suitable for some DNA and protein separations. Volatile. Should be used fresh.
8.0	0.1 M Tris/HCl 1 mM EDTA	Very good solubility for DNA and RNA.
8.6	6 M Guanidine HCl + 50 mM Tris/HCl	Good UV-transparency. Suitable for large fragments of proteins which can be dialyzed remove the guanidine.
11.5	0.05 M NaOH Acetonitrile 30% + suitable buffer 6 M Guanidine HCl 0.1% SDS, Tween [™] or similar	Good solubility for some compounds. For separation of very hydrophobic compounds. Volatile. Molecular weight determinations. Good solubility for some proteins e.g. membrane proteins. Make sure to equilibrate completely with the detergent solution.

Note: The buffers recommended for MonoQ[®], MonoS[®] and MonoP[®] can be convenient to use for the gel filtration step.

[™] Tween is a registered trademark of Merck

Running conditions for best results

Flow rate optimisation

The optimum separation is generally obtained with a flow rate of 0.3-0.5 ml/min, but an optimisation is recommended for your specific sample and conditions.

Fast runs

Fast runs are recommended only for Superose 12. Such a run is best performed at a flow rate of 1.0 ml/min. In this range, the resolution is generally sufficient for small molecules and for group separations.

Efficient separation of large molecules

Large molecules, with low coefficients of diffusion, often require lower flow rates, 0.1-0.3 ml/min.

Loading capacity

For the highest resolution, volume of 0.1-1.0% (24-240 µl) of V_c is recommended. Moreover the sample should not contain more than 5 mg of protein.

Eluent system

To avoid ionic interactions, usage of 0.05 M phosphate buffer with 0.15 M NaCl, pH 7.0 is recommended, or buffer with the same ionic strength. The eluent for the gel filtration run can often be chosen to simplify a later separation stage, for example, the gel filtration column can be equilibrated and eluted with the start buffer for a subsequent ion exchange separation.

Two columns in series

Separations can be improved by using two columns in series (connected using the Union, M6 female/M6 female, Code No. 18-3856-01). For two columns, reset the pump pressure limit control to 2.5 MPa (25 bar, 360 psi) for Superose 6 and 4 MPa (40 bar, 580 psi) for Superose 12. If you connect more than two columns of Superose 6 or 12 in series, do not exceed 4 MPa.

Molecular range determinations

We recommend the following proteins for calibration: thyroglobulin, ferritin, IgG, aldolase, albumin, ovalbumin, β -lactoglobulin, myoglobin, cytochrome c.

Increased back-pressure and column cleaning

Clean your column if you observe:

- an increased back-pressure
- a gap that has become visible between adaptor and filter
- a colour change at the top of the column
- a loss of resolution

The following steps should be performed in sequence (do not reverse the flow during cleaning since this may cause a loss of efficiency):

1. Change the filter at the top. Instructions for changing the filter are supplied with the Filter kit.
2. Set the pressure limit control at 1 MPa (will not allow a flow rate of more than 0.3-0.5 ml/min) for Superose 6 and 2 MPa for Superose 12.
3. Wash with 25 ml 50% acetic acid.
4. Wash with 25 ml water.
5. Wash with 25 ml 20% ethanol (run at a low flow rate).
6. Wash with 25 ml water.
7. Wash with 25 ml 0.1 M NaOH.
8. Rinse with 25 ml water and a few injections of 50% acetic acid (3 x 200 µl).
9. Equilibrate with buffer.

If you have used **detergents**, rinse with 50 ml water and perform step 5.

A cleaning volume of 25 ml is only a guideline. The practical requirements are best determined by monitoring the baseline, which should be stable at the end of each step.

Checking the column packing

A well packed column is essential for high performance, so periodic packing checks are recommended. Cytochrome c (Sigma type 3) is a good test protein since it is coloured and readily available. Apply 100 µl sample containing 1-2 mg protein/ml in 10 mM phosphate buffer, pH 7.0. Perform a

normal run. If the band is wavy, diffuse, or not horizontal, perform the following steps in sequence until the column efficiency has improved.

- Make sure there is no space between the top adaptor and the gel bed. If there is, a filter change is probably needed. To adjust the adaptor, disconnect the inlet tubing from the valve and rotate the **red** adjusting ring clockwise until there is no visible space between the gel and the adaptor. Finally rotate the red ring an additional turn. Reconnect the inlet.
- Change the filter on the top of the gel bed and clean the column. Instructions for changing the filter are supplied with the Filter kit.
- Reverse the flow direction in the column; this may distort the packing and should only be used as a last resort.

Efficiency test

After column maintenance procedures the efficiency of the column should be checked. Column efficiency, expressed as plates per metre (H^{-1}), is estimated using following equation:

$$H^{-1} = 5.54 * (V_R/w_h)^2 * (1000/L)$$

L = bed height (mm)

V_R = retention (elution) volume

w_h = peak width at half peak height (ml)

H^{-1} = number of theoretical plates/metre

Sample: 100 µl of acetone (p.a.), 5.0 mg/ml

Eluent: Milli Q water

Flowrate: 1.0 ml/min

Detector: UV-M, 280 nm, 0.5 AUFS

Chart speed: 1.5 cm/min

The number of theoretical plates per metre (H^{-1}) should be more than 30 000.

Function test

An alternative to the efficiency test to check column performance is the function test described here.

Function test, Superose 6 HR 10/30

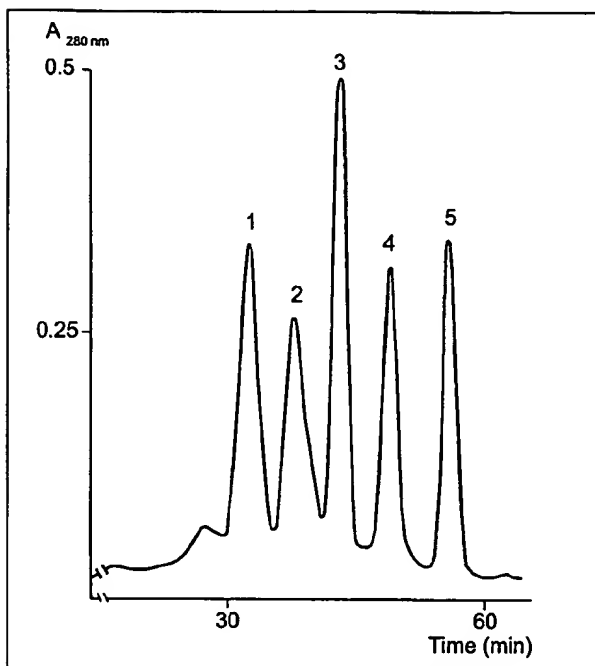


Fig. 1. Typical chromatogram from a function test of Superose 6 HR 10/30.

Function test Superose 12 HR 10/30

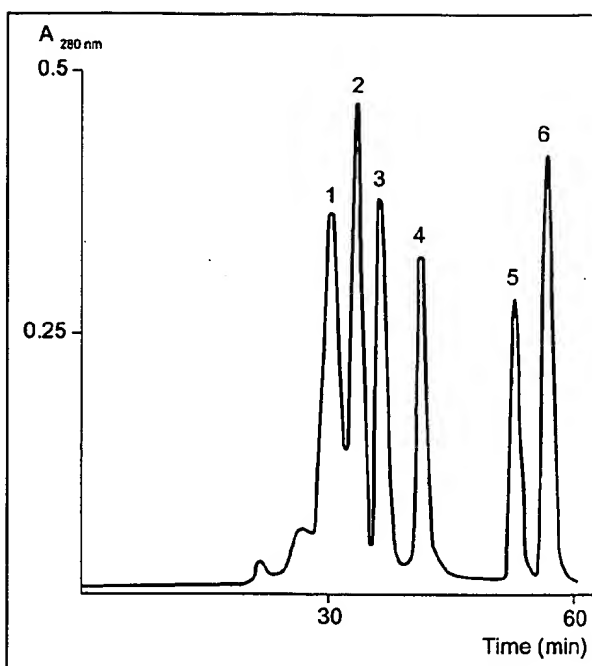


Fig. 2. Typical chromatogram from a function test of Superose 12 HR 10/30.

Experimental:

Sample: 100 μ l solution containing

1. Thyroglobulin (M_r 669 000), 5 mg/ml
2. Ferritin (M_r 440 000), 0.3 mg/ml
3. Bovine serum albumin (M_r 67 000), 8 mg/ml
4. Ribonuclease A (M_r 13 700), 5 mg/ml
5. Glycyl tyrosin (M_r 238), 0.6 mg/ml

Buffer: 0.05 M phosphate buffer with 0.15 M NaCl, pH 7.0

Flow rate: 0.4 ml/min

Detector: UV-M, 280 nm, 0.5 AUFS

Chart speed: 0.5 cm/min

Experimental

Sample: 100 μ l solution containing

1. IgG (M_r 160 000), 2.5 mg/ml
2. BSA (M_r 67 000), 8 mg/ml
3. b-lactoglobulin (M_r 35 000), 2.5 mg/ml
4. Cytochrome C (M_r 12 400), 1 mg/ml
5. Vitamin B₁₂ (M_r 1355), 0.1 mg/ml
6. Cytidine (M_r 243), 0.1 mg/ml

Buffer: 0.05 M phosphate buffer with 0.15 M NaCl, pH 7.0

Flow rate: 0.4 ml/min

Detector: UV-M, 280 nm, 0.5 AUFS

Chart speed: 0.5 cm/min

Storage and prevention of microbial growth

Store your column in 20% ethanol. Avoid prolonged exposures to extremes of pH.

Before storage, connect the rubber tubing supplied with the column between the inlet and the outlet of the column. The tubing should be filled with liquid. This will prevent damage of the gel packing due to temperature variations during storage.

Further information

For more technical information on Superose please contact your local Amersham Biosciences representative.

Products and accessories

Precision Columns

Precision columns available. The column range is being expanded.

Column	Code No.
<i>Ion exchange:</i>	
Mini Q™ PC 3.2/3	17-0686-01
Mini S™ PC 3.2/3	17-0687-01
Mono Q® PC 1.6/5	17-0671-01
Mono S® PC 1.6/5	17-0672-01
<i>Gel filtration:</i>	
Superose® 6 PC 3.2/30	17-0673-01
Superose® 12 PC 3.2/30	17-0674-01
Superdex® 75 PC 3.2/30	17-0771-01
Superdex® 200 PC 3.2/30	17-1089-01
Superdex Peptide® PC 3.2/30	17-1458-01
Fast Desalting PC 3.2/10	17-0774-01
<i>Reversed phase:</i>	
µRPC C2/C18 PC 3.2/3	17-0703-01
µRPC C2/C18 SC 2.1/10	17-0704-01
Sephasil™ C8 SC 2.1/10	17-0769-01
Sephasil™ C18 SC 2.1/10	17-0904-01
<i>Hydrophobic interaction:</i>	
Phenyl Superose® PC 1.6/5	17-0772-01
<i>Affinity chromatography:</i>	
NHS-activated Superose® PC 3.2/2	17-0773-01

Other products

Products	Code No.
Sample loop 5 µl	18-1800-70
Sample loop 10 µl	18-1800-71
Sample loop 20 µl	18-1800-72
Sample loop 50 µl	18-1800-73
Sample loop 100 µl	18-1800-74
Sample loop 200 µl	18-1800-90
Sample loop 500 µl	18-1800-91
Sample loop 1000 µl + 2000 µl	18-5897-01
Union M6 female/1/16" male	18-3858-01
Superloop 10 ml	19-7585-01
Superloop 50 ml	19-7850-01
Superloop 150 ml	18-1023-85
Prefilter 0.8 mm	18-1800-75
Prefilter 3.2 mm	18-1800-76
Precision Column Holder	17-1455-01

Spare parts for the column

Products	Code No.
Protective caps (2/pk)	18-1015-28
1. Top assembly HR 10	18-1541-01
2. Bottom assembly HR 10	18-1542-01
3. Tubing connectors*	19-7476-01
Flanging/Start Up kit	
120 V	19-5079-01
220 V	19-5090-01

* You need the flanging/Start Up kit to attach new tubing connectors

